HOMOPLASTIC GRAFTING IN STENTOR COERULEUS

PAUL B. WEISZ

Arnold Biological Laboratory, Brown University, Providence, R. I.

Grafting techniques applied to the study of cell aggregates have long been among the most powerful tools of the experimental biologist. For technical reasons, similar procedures have not been generally applicable to single cellular units, but the desirability of such methods is evident.

Among ciliates, fusion complexes made up of two or more individuals are not new. In an important paper, Fauré-Fremiet (1945) reviews most of the known cases. He shows that such complexes either are found fortuitously in mass cultures, or can be produced by experimental or bacterial interference with the fission process, a procedure which leaves the daughter individuals joined together. Deliberate grafting has not yet been described, probably because physico-chemical peculiarities of the protoplasm in most ciliates generally preclude successful fusion of individuals by experimental techniques now available.

The feasibility of grafting in the heterotrich *Stentor coeruleus* has first been pointed out by Tartar (1941). He finds that "if two cells are cut and then immediately pressed together . . ., the two will fuse as one." Further exploration by the present writer has led to the development of a relatively simple method which permits the controlled, oriented fusion of two or more whole Stentors, or of specific fragments derived from several parent organisms.

Beyond the description of the methods employed, the aim of this paper is to set forth the broad principles which have been found to underlie grafting phenomena in *S. coeruleus*. Particular attention will be given to: the circumstances in which stable or unstable fusion complexes are formed, following the union of different portions of ectoplasm, endoplasm, and nuclei from two or more parent Stentors in several possible combinations and orientations; the reorganizational processes which can be observed in differently constituted fusion complexes; and the morphogenetic mechanisms which are operative in these phenomena.

Materials and Methods

Stentors were selected from cultures maintained in this laboratory for several years. The animals were fed intensively so that large specimens (up to 350 micra in diameter) were available for experimentation. Preliminary attempts at grafting had shown that any successful technique must permit (a) the maintenance of a desired orientation and contiguity of two whole animals or fragments for at least 30 to 40 seconds; and (b) the simultaneous rupturing of the apposed pellicles and ectoplasmic layers, so that the exposed endoplasms can flow out and fuse before the formation of surface precipitation membranes. The latter normally appear within 1 or 2 seconds.

The procedure finally adopted permitted the preparation of fusion complexes of any desired volume or specific constitution, and did not involve any loss of protoplasm during the operation. Several drops of a viscid suspension of methyl cellulose in water were spread on a glass slide, in a layer approximately 1 to 2 mm thick. A small drop of water containing two Stentors was pipetted into this layer. By manipulation with needles under a dissecting microscope, the animals were pushed gently into the methyl cellulose until they came to rest side by side. If the methyl cellulose had proper consistency, locomotion of the animals was prevented, and they could be maintained in a desired orientation and in continuous contact for as long as 1 minute or more. Sharp steel needles were used to rupture the apposed surfaces. When properly done, fusion was usually accomplished at the first attempt. If necessary, repeated ruptures could be made until the graft had taken. The fusion complex, along with a minimum of methyl cellulose, was transferred to culture water and the double organism was carefully freed from the embedding medium. After waiting 20 to 30 minutes for the fused organisms to heal firmly together, further operative steps could be undertaken. Doublets could be joined to yield multiple fusion complexes; specific differentiated organelles could be excised from one or both of the units; macronuclei of either of the components could be removed wholly or partially; or any combination of these procedures could be carried out. If small fragments were to be grafted to whole animals or to other fragments, two intact Stentors were first joined, and the required excisions were performed later.

Homopolar Doublets

The long axes of organisms constituting such doublets are oriented in the same direction and in the same sense. Categories 1 to 4, below, comprise experiments on parabiotic homopolar complexes, while category 5 deals with telobiotic homopolar doublets. In the former, the results are the same whether the units are joined in ventral-to-ventral, dorsal-to-dorsal, lateral-to-lateral, or any other combination. Analogously, in telobiotic doublets it is immaterial to what extent the units are rotated relative to each other. The following descriptions are based on a study of at least three cases in each type of experiment.

1. *Parabiotic homopolar fusion; no other experimental modification.* No visible changes occur for the first 4 to 6 hours. The two macronuclear chains are distinct and lie approximately parallel. Both peristomes and both holdfasts remain normal and functional, and both contractile vacuoles persist, maintaining, however, an independent rhythm of pulsation. Irritation of one member of the fused pair leads to smooth simultaneous contraction of both members, indicating an early functional integration of neural and contractile organelles.

Changes are first noticeable in the holdfasts. Approximately 5 to 6 hours after fusion, one of them decreases in size. If the components of the doublet are of unequal volume, the foot of the smaller member is invariably affected. The reduced foot later loses its adhesive properties, becomes permanently retracted, and is finally resorbed completely. Twelve to 18 hours after fusion, one of the contractile vacuoles and one of the oral regions (again the smaller structure, in each case) begin to dedifferentiate. In the oral area the gullet disappears first, and in the ensuing hours the entire peristome is gradually resorbed. Some 24 hours

after fusion, the original doublet has the appearance of a large single Stentor, although the two macronuclear chains are still distinct. Within a further 24-hour interval physiological reorganization occurs (*cf.* Weisz, 1949a), a process which results in the replacement of the persisting oral region by newly differentiated oral organelles, and in the transformation of the macronuclei into a single chain composed of proportionately larger nodes. A normal single individual is thus reconstituted. Vegetative division generally takes place within a short time.

In its essential features, this reorganizational sequence is identical with that described by Balbiani (1891), Johnson (1893), and Weisz (1951) for doublets of *S. coeruleus* found in mass cultures, or produced experimentally by interference with the process of fission. The sequence differs fundamentally from that in certain homopolar doublets of a variety of ciliates, *e.g., Urostyla weissei* (Fauré-Fremiet, 1945), or *Euplotes patella* (Kimball, 1941), in which the double organization is stable, and may give rise to two doublets by fission.

2. *Parabiotic homopolar fusion; excision of one or both of the systems of oral organelles, or one or both of the holdfasts.* If one of the oral regions is removed, regeneration does not occur. This holds true regardless of the initial relative size of the region. The foot of the gullet-less member is resorbed some 6 hours after fusion, while the contractile vacuole persists for 12 hours or more. Complete reorganization to a single individual, including the formation of a single macronuclear chain, is accomplished 24 hours after the operation. In effect, experimental removal of one oral region leads to a process of resorption similar to that described in No. 1, except that it is more rapid. Suppression of the gulletless member is always involved, regardless of its initial relative volume.

Analogous results are obtained if one of the holdfasts is excised. Regenerative replacement never occurs, and the persisting foot becomes the definitive holdfast of the reorganized single individual.

If both oral regions are cut off, only a single region ever regenerates. Using surface peculiarities and macronuclear configurations as identifying criteria, it has been found that the regenerating region is always part of the larger member of the fused pair. In the non-regenerating unit the foot, and later the contractile vacuole, are resorbed. As above, a single individual is reconstituted within 24 hours after fusion. Results are analogous if both holdfasts are removed, *i.e.*, only a single foot regenerates.

3. *Parabiotic homopolar fusion; complete removal of one macronucleus.*

a. Two whole animals are joined and all macronuclear nodes of one member are excised one by one; all except two or three nodes of the other member are similarly excised. (Enucleation procedures disrupt the constitutents of the endoplasm considerably. After one or two nodes have been excised, it is virtually impossible to ascertain the original location of most of the remaining nodes. Fortunately, two or three nodes just underneath the peristome field usually are not as easily dislodged as others. By removing all but these in one member, and all nodes in the other member, it is possible to prepare a system in which all macronuclear substance reliably derives from only one of the members. It is already known (Weisz, 1949b) that even a single anterior node is functionally equivalent to a whole chain of nodes, at any cycle stage.)

Doublets of this type ultimately reorganize into single individuals. The two or three nodes soon split up into smaller nodules. Each of these gradually in-

creases in size until a fairly normal macronuclear chain has re-formed. In other respects, the reorganization sequence parallels that observed in No. 1, and lasts approximately one to two days. In this reorganization, it is always the individuality of the enucleated unit which is suppressed.

b. A normal whole animal is joined with an animal undergoing physiological reorganization, at a stage when its nucleus is condensed maximally. The condensation nuclei never number more than three and are easily distinguished from the nodulated chain of the normal partner. Excision of these nuclear masses presents little difficulty, and at the same time the operation ensures that the entire nucleus, and only this nucleus, is removed. At the stage of maximal nuclear condensation, the oral region of the reorganizing member is only partially redifferentiated. The new peristome band is incompletely developed, and is not as yet fully aligned in its presumptive anterior position; a new gullet has not yet formed (*cf.* Weisz, 1949a).

Such doublets also reorganize into single individuals within one to two days. The incomplete oral region of the enucleated member never develops further, and begins to be resorbed after a few hours. In other respects the results are the same as in No. 3a.

4. *Parabiotic homopolar fusion: an animal undergoing physiological reorganization (condensation nuclei excised) joined to normal animal (oral region cut off).* Regenerative replacement of the excised oral region never occurs. On the other hand, the incomplete oral apparatus of the enucleated unit develops further and gives rise to a normally constituted peristome field and to a new gullet. These organelles become the definitive oral region of the single individual which is modelled from the components of the original doublet. Reorganization is usually complete after 24 hours.

5. *Telobiotic homopolar doublets.* Such doublets are produced by removing the foot of one organism and the oral region of another, and grafting the cut surfaces together. In effect, they represent large single individuals from the outset. Apart from the resorption of the contractile vacuole of the posterior unit, other reorganization phenomena cannot be observed. Experiments on regeneration and enucleation have been carried out. The results are wholly equivalent to those obtained in normal, single animals.

Heteropolar Doublets

The majority of experiments under this heading deal with telobiotic complexes, but parabiotic ones are also included. Telobiotic doublets in categories 6 to 9 are foot-to-foot heteropolar; they were produced by removing the holdfasts of two whole animals and grafting the cut surfaces together. Doublets in category 10 are mouth-to-mouth heteropolar. For each category, the results are the same, regardless of the relative degree of rotation of the units around their common axis. If this axis does not form a straight line from the outset, heteropolarity is never maintained. An originally obtuse angle between the components of the doublet becomes progressively more acute and, within three hours at the most, a homopolar parabiotic system has formed. Thus if necessary, varying amounts of lateral cytoplasm must be excised after fusion, until the doublet acquires a straight longitudinal axis. The following descriptions are based on a study of at least three cases in each type of experiment.

6. *Telobiotic (or parabiotic) heteropolar fusion; no other experimental changes.* Differentiated organelles do not undergo reorganizational changes, and both oral regions persist. Approximately 7 to 12 hours after fusion, a constriction becomes noticeable along the ectoplasmic suture line between the two components. The suture line is defined by the abrupt discontinuity and the derangement of the surface stripes where the components join. A similar suture is recognizable for many hours in homopolar grafts, but signs of constriction never appear in these. In heteropolar grafts, the constriction deepens progressively and 24 hours after fusion, only a slender strand may still connect the two members. Complete separation of the members occurs invariably, the points of last contact developing into new holdfasts.

In a given member, the number of macronuclear nodes at the time of constrictive separation is generally either greater or smaller than before fusion, although the total number of nodes in the doublet remains constant. Also, the volumes of the separated units may be strikingly different from their volumes before fusion, even allowing for the fact that small quantities of cytoplasm may have been excised during the grafting procedure. The constriction evidently cuts through the interior protoplasm regardless of whether nuclei and endoplasm in the constriction plane derive from one or the other component. It may be concluded that the plane of constriction is determined solely by the ectoplasmic suture line. The phenomenon of constructive separation is strikingly reminiscent of vegetative division, a process in which constriction takes place along a well-defined ectoplasmic fission line (Weisz, 1951).

A similar result has been described by Lund (1917) for heteropolar doublets of *Bursaria truncatella.* In this species, however, constrictive separation appears to occur only if the units are of equal volume; if they are not, "the smaller and weaker member sooner or later dedifferentiates and the whole or part of its substance becomes part of the stronger and larger member" (by redifferentiation with a reversed polarity). In *S. coeruleus,* on the other hand, constrictive separation takes place regardless of the initial relative volume of the units in the doublet, and none of the organelles ever dedifferentiate. (Moreover, reversal of polarity through de- and redifferentiation has never been observed in Stentor.) In other species studied, a heteropolar configuration is either remodelled into a homopolar one, or the doublet disintegrates within a short time (*cf.* Fauré-Fremiet, 1945).

7. *Telobiotic (or parabiotic) heteropolar fusion; removal of one or both oral regions.* In either case, normal regeneration occurs. Regeneration is completed long before the units separate. If the oral areas are excised only one to two hours before the units are expected to separate, they divide as fragments and regenerate thereafter.

8. *Telobiotic heteropolar fusion; excision of one macronucleus.*

a. Two whole animals are joined, and by methods described in No. 3a, all except 2 or 3 macronuclear nodes of one of the components are excised. Under these conditions, the heteropolar configuration is never maintained, even if all possible precautions are taken to produce an initially truly heteropolar doublet possessing a straight axis. Transformation into a homopolar parabiotic system ensues invariably within a few hours. Thereafter, the course of events is identical with that described in No. 3a.

If one of the units is not completely enucleated and even a single node is retained, heteropolarity persists and constrictive separation takes place.

b. A normal Stentor is joined with one undergoing physiological reorganization, and the condensation nuclei of the reorganizing member are excised. As in No. 8a, transformation into a homopolar doublet soon takes place. Thereafter, a single individual arises by processes described in No. 3b.

9. *Telobiotic heteropolar fusion; an organism undergoing physiological reorganization (condensation nuclei excised) joined to normal animal (oral region cut off).* A homopolar doublet soon forms as above. Subsequent reorganization follows the steps outlined in No. 4.

10. *Mouth-to-mouth heteropolar doublets.* Such complexes are produced by removing the oral regions of two whole animals and grafting the cut surfaces together. As in foot-to-foot heteropolar doublets, heteropolarity changes to homopolarity unless the doublet possesses a straight longitudinal axis.

Regenerative replacement of both oral regions begins four to five hours after fusion, the expected time interval at room temperature. Some 12 hours after fusion, regeneration is completed. Normal, well-formed peristomes and gullets have developed in each component. These oral organelles are situated laterally, along the suture line of the doublet. Constrictive separation does not take place. Twenty-four hours after fusion, the doublet has become remodelled into a homopolar parabiotic system, the two holdfasts now lying side by side, and the two oral regions occupying the anterior aspect of the doublet. Further reorganization into a single individual occurs as in No. 1.

Multiple Complexes

By grafting doublets to other doublets or to single animals, a considerable number of Stentors can be combined in any desired pattern. Complexes consisting of up to 6 individuals have been prepared, each complex representing a random or non-random pattern of homopolar or heteropolar grafts. The results may be summarized as follows.

a. In properly formed complexes, each component occupies a definite continuous space within the system. The space is initially identifiable by a virtually intact system of surface stripes, by a distinct chain of macronuclear nodes, and by normally functional oral organelles. Such complexes are viable, regardless of the orientation of the components.

The grafting procedure sometimes leads to a severe disruption of the surface organelles of the fusion complex. As a result, the spatial individuality of given units is destroyed more or less completely. This effect can also be achieved intentionally, by passing the operating needles rapidly and at random through the fusion complex. Such systems are not viable. Abortive attempts at surface reorganization are noticeable, but after some days vacuolation becomes increasingly apparent, and the complex ultimately disintegrates.

b. During the first 12 to 18 hours after fusion, more or less extensive ectoplasmic shifts and internal cyclosis-like rearrangements occur in viable complexes. Regardless of the initial fusion pattern, these shifts change the pattern into one of three characteristic types. In one of these, the system acquires a single major axis, and all units are aligned in parabiotic homopolar orientation. In a second

type, the complex is essentially biaxial. The two axes form a straight line, but have opposite sense, *i.e.*, the two oral poles are at opposite ends of the straight line, and the two pedal poles join at a common point, approximately mid-way along the line. Around each of these axes are grouped one or more units in parabiotic homopolar combination. These homopolar groups are consequently telobiotic and heteropolar to each other. In a third, less common, pattern of organization, the complex is triaxial. The three axes radiate out from a common (pedal) center and subtend roughly equal angles. One or more units are grouped around each of the axes in homopolar parabiotic combination.

The number of sub-units composing the complex does not influence the type of pattern emerging after 24 hours. Rather, a given pattern is partly an enhanced expression of a fundamental initial architecture of the complex, and partly a result of form-regulating rearrangements. The latter are in turn predictable from the spatial peculiarities of the fusion mass. Experiments indicate that the three patterns described are probably the only ones which can possibly emerge. Attempts to create radial tetraxial complexes always meet with failure. In such attempts, adjacent axes at best subtend 90-degree angles, which eventually increase to 180 degrees or to zero, bringing the units grouped around these axes into heteropolar or homopolar alignment. Thus, even if all four axes lie in one plane, the second pattern described above emerges. Random polyaxial and completely irregular complexes also transform into one of the organizations described.

c. A homopolar parabiotic group, whether it represents an entire complex or only a part, ultimately reorganizes to a single organism or sub-unit, by steps described in principle under experiment No. 1. Oral regions are dedifferentiated one by one, at a rate of approximately one every 24 hours. The last oral region persists.

Parabiotic homopolar groups which are telobiotic and heteropolar to each other separate by constriction after one or two days. This holds true for biaxial as well as for triaxial complexes. The latter, if not previously transformed into homopolar groups, may either split into three monaxial subcomplexes at roughly the same time, or may first give rise to one monaxial and one biaxial complex.

d. If one or more of the organisms which are originally grafted together are in late stages of the vegetative cycle, fission will occur while they are joined to the complex. Depending on the manner of fusion, the ectoplasm in the presumptive path of the fission line may either be partially excised or twisted out of position, or may lie entirely free and unobstructed. In the former case, fission cannot be completed (*cf.* Weisz, 1951). A new oral area and a (partial) posterior daughter individual are formed nevertheless, thus increasing by one the number of units in the complex. In the latter case, fission is completed and the anterior (or the posterior) daughter individual is constricted off from the fusion mass.

Discussion and Conclusions

The following general conclusions can be drawn from the data.

1. Grafting procedures in *S. coeruleus* yield fusion complexes which are invariably labile. A given complex, if viable, always reorganizes into one or more single individuals: (a) by breaking up into units through constriction, in which

case the constituent organisms of the complex are recovered more or less *in toto;* or (b) by stepwise dedifferentiation of all but one of the component units, in which case a single, enlarged but normal individual develops; or (c) by a combination of these two processes. Viability of the complex is contingent upon the retention of the essential individuality of each unit, particularly as regards the surface organelles. If the ectoplasm is too severely disrupted, the fusion mass ultimately disintegrates.

2. It is clear that the instability of fusion complexes cannot be due to either endoplasmic or nuclear incompatibility. This is substantiated both by the fact that grafting is possible at all, and by the observation that all homopolar and many heteropolar complexes give rise to individuals which contain nuclei and endoplasm from more than one source, in permanent, stable union. The lability of fusion complexes and the different experimental results must therefore be interpreted through ectoplasmic activities.

It has been shown elsewhere (Weisz, 1951) that in *S. coeruleus* all phases of morphogenesis decisively involve kinety I. According to Lwoff (1950) and his collaborators, this and every other kinety is a system of ectoplasmic organelles composed of a longitudinal row of granules, the kinetosomes, and a longitudinal fiber, the kinetodesma, which is situated to the right of the kinetosomes. Kineties are thus asymmetrical, being polarized both transversely and longitudinally. Since present experiments deal primarily with problems of morphogenesis, the results should be interpretable in terms of kinetal function.

3. Stepwise dedifferentiation of supernumerary units constitutes the method of reorganization in complexes composed of units which are initially or secondarily joined in homopolar and parabiotic orientation. In any homopolar association of organisms, all kineties are oriented in the same sense and in the same direction, *i.e.,* they are mutually homopolar themselves, both longitudinally and transversely. Thus, the net effect of this type of grafting is the interpolation, into an existing layer of kineties, of another section of ectoplasm containing identically oriented kineties. No fundamental change has occurred in kinetal orientation *per se.* The graft is integrated functionally and remains permanently in place.

On the other hand, the procedure introduces additional kineties I into the system, and an equal number of oral organelles, holdfasts, and contractile vacuoles. Why is stability and an equal status of "dominance" not retained by all of the sets of organelles and the kineties I? Indeed, an essentially identical question must be raised for any normal single Stentor: how are individual kineties prevented from exercising the same specialized functions as kinety I, particularly since it has been shown (Weisz, 1951) that all kineties are fundamentally equipotential? No fully conclusive answer can as yet be given to this important general problem. Ectoplasmic structures arise from, and are maintained by, specialized kinetosomes which in turn are derivatives of the kinetosomes in kinety I (*cf.* Weisz, 1951). It is clear, therefore, that all questions of physiological dominance involving differentiated organelles or kineties reduce to problems of kinetosomal dominance.

Present experiments and certain aspects of earlier observations on kinetal processes suggest a metabolic solution of the problem of kinetosomal dominance. If given kinetosomes were to possess, or could acquire, a competitive metabolic advantage over other kinetosomes, the morphogenetic potentials of the latter would remain or become inhibited. The experiments indicate that kinetosomes of the

largest sub-unit in a fusion complex generally acquire dominance over kinetosomes in other sub-units. This might imply that particular dominance relationships within the complex are due to quantitative differences of structure, and thus presumably of maintenance metabolism, among the sub-units. Dedifferentiation of the organelles in the smaller sub-units, and loss of specialized function of their kineties I, would then be a consequence of continuous successful competition by the kinetosomes of the sub-unit possessing a quantitative metabolic advantage.

Furthermore, kinetosomes of complexly differentiated organelles are dominant over kinetosomes of less complexly differentiated structures. In normal Stentors, for example, oral kinetosomes are dominant over those in kinety I: the latter obviously can not produce additional oral regions as long as one is already present, but can do so as soon as the oral kinetosomes are removed (Weisz, 1951). This relationship accounts for the observation (experiment No. 2, above) that in the absence of the larger oral region in a doublet, the smaller oral region becomes dominant over kinety I of the larger sub-unit, and regeneration of the excised structures cannot occur. In the absence of both oral regions, however, kinety I of the larger sub-unit becomes dominant over all other kineties, and this kinety alone gives rise to a new oral region. Analogous interrelationships hold true between kineties and holdfasts or contractile vacuoles, as the experiments indicate. In view of the fundamental equipotentiality of kinetosomes, dominance relationships in the above instances might again imply intrinsic, or micro-environmentally conditioned, metabolic specializations of kinetosomes. These specializations would determine the degree of complexity to which given organelles become differentiated.

4. In heteropolar complexes, the predominant method of reorganization entails constrictive separation (unless the system transforms secondarily into a homopolar complex). In these instances, kineties are oriented in the same direction, but in the opposite sense, *i.e.*, they are longitudinally and transversely heteropolar. Comparison with homopolar grafts indicates that the lability of these complexes is due primarily to the heteropolarity of the apposed kineties. As in other aspects of kinetal behavior (Weisz, 1951), properties of a magnet are suggested, inasmuch as like poles of the kineties appear to repel each other. Consequently, each unit in the complex should, and does, retain complete ectoplasmic individuality (even though an early neural and contractile integration is indicated). Dominance relationships are not established, and each unit therefore retains the essential characteristics of a free, single individual. This includes the capacity of regeneration, maintained by an independently functional kinety I (*cf.* experiment No. 7). Constriction itself, considered from the standpoint of a single unit, is a process equivalent to that following removal of the posterior region in a normal free animal: the region of the cut elongates and narrows down to a protruding tip, the latter developing into a new foot. As already noted, fissional constriction proceeds through very similar steps; the contrictional forces involved are probably the same.

5. The experiments in No. 3, No. 4, No. 8, and No. 9 show that macronuclei are cross-active from one unit to another. Nuclei in one unit not only maintain viability and normal function of organelles in an enucleated unit, but also support differentiation, *e.g.*, the completion of oral differentiation in the enucleated member in experiment No. 4. On the other hand, enucleation evidently does affect the status of dominance of a unit within a complex. Even though the enucleated unit

of a parabiotic doublet may be the larger component, the smaller nucleated member will nevertheless acquire dominance (*cf.* experiment No. 3a). Macronuclear cross-activity thus cannot fully compensate for the absence of nuclei in a unit. It is impossible to determine the reasons for this from the existing evidence, but since the question of dominance is involved, a solution in metabolic terms may again be indicated. Comparison of experiments No. 3b and No. 4 also shows that loss of the macronucleus does not affect the status of dominance as much as loss of the oral apparatus.

6. Secondary transformation of a heteropolar doublet into one which is parabiotic and homopolar occurs: whenever the two axes do not form a straight line; when one of the units is enucleated; and in mouth-to-mouth heteropolar associations.

Biaxial systems in which the two axes do not have the same direction are fundamentally monaxial from the outset. A major axis bisects the angle between the components and such systems are actually parabiotic, the area of fusion being confined to the most posterior regions. Subsequent reorganization merely makes the parabiosis more obvious and effects a more nearly homopolar orientation of the kineties.

An originally heteropolar doublet with an enucleated component represents a system in which the components are no longer co-equal. Despite macronuclear cross-activity, the enucleated unit loses an as yet indefinable characteristic of kinetal individuality (*cf.* preceding section), and a dominance relationship presumably arises within the complex. It is not apparent, however, how these conditions could lead to the initial change of kinetal polarity.

Transformation of mouth-to-mouth heteropolar doublets also remains unexplained. Such complexes maintain true heteropolarity until the two oral regions have fully regenerated. Only then does an orientational change occur. Present evidence does not show why constrictive separation does not take place instead.

Regardless of the specific conditions which induce orientational changes in given fusion complexes, the mechanism of transformation appears to be the same in every case. Unilateral resorption and a consequent shortening of kineties is probably involved.

7. While many of the results merely raise new problems, a sufficient body of evidence has accumulated to indicate that grafting phenomena in *S. coeruleus,* like fusion phenomena in other species (Fauré-Fremiet, 1945), are governed to a great extent by kinetal and kinetosomal processes. Kineties and kinetosomes should therefore hold clues to questions as yet unresolved. Among the most puzzling of such questions is the different behavior of fusion complexes in different species. Further work, conceivably supplemented by grafting techniques, may not only yield answers to this problem, but may also increase significantly our understanding of kinetal and kinetosomal function.

Summary

1. A technique is described which permits oriented grafting of two or more whole Stentors or fragments.

2. Fusion complexes in *S. coeruleus* are invariably labile. If the surface organelles of the component units are too severely disrupted, the complex disintegrates.

In viable homopolar systems, all but one of the units dedifferentiate, and a single enlarged individual is formed. In heteropolar systems, the units maintain their individuality and finally constrict apart, in a process strikingly reminiscent of fissional constriction. Random multiple complexes first acquire one of three characteristic patterns of organization, and then reorganize into single organisms by either or both of the methods mentioned.

3. In a homopolar parabiotic doublet, the unit which later dedifferentiates is always the smaller component. It cannot regenerate excised parts, even before its individuality is suppressed. On the other hand, if the oral organelles of the larger component are excised, they do not regenerate, and the smaller unit becomes dominant. In heteropolar complexes, each unit can regenerate excised parts, regardless of its initial relative volume.

4. Macronuclei are found to be cross-active, nodes of one unit maintaining normal function and the capacity of differentiation in an enucleated unit. However, the particular status of dominance of a unit is abolished by enucleation.

5. Questions raised by the experiments are shown to be reducible to problems of kinetal and kinetosomal function. As far as is possible, therefore, the results are interpreted in terms of activities of kineties and kinetosomes, ectoplasmic organelles known to be decisively involved in processes of ciliate morphogenesis.

LITERATURE CITED

Balbiani, E. G., 1891. Sur la formation des monstres doubles chez les Infusoires. *J. de l'Anat. et Physiol.*, **27**: 169–196.

Fauré-Fremiet, E., 1945. Symetrie et polarité chez les Ciliés bi- ou multicomposites. *Bull. Biol. Fr.-Belg.*, **79**: 106–150.

Johnson, H. P., 1893. A contribution to the morphology and biology of the Stentors. *J. Morph.*, **8**: 467–562.

Kimball, R. F., 1941. Double animals and amicronucleate animals in *Euplotes patella*, with particular reference to their conjugation. *J. Exp. Zool.*, **86**: 1–32.

Lund, E. J., 1917. Reversibility of morphogenetic processes in Bursaria. *J. Exp. Zool.*, **24**: 1–34.

Lwoff, A., 1950. Problems of morphogenesis in Ciliates. Biol. Res. Series, J. Wiley & Sons, New York.

Tartar, V., 1941. Intracellular patterns in the morphogenesis and regeneration of ciliate protozoa. *Growth*, **5** (Suppl.): 21–40.

Weisz, P. B., 1949a. A cytochemical and cytological study of differentiation in normal and reorganizational stages of *Stentor coeruleus*. *J. Morph.*, **84**: 335–364.

Weisz, P. B., 1949b. The role of specific macronuclear nodes in the differentiation and the maintenance of the oral area in Stentor. *J. Exp. Zool.*, **111**: 141–156.

Weisz, P. B., 1951. An experimental analysis of morphogenesis in *Stentor coeruleus*. *J. Exp. Zool.*, (in press).